2.0 METHODS

2.1 Source of Study Fish

All chinook salmon used in the study were of hatchery origin. Fish were obtained from Little White Salmon National Fish Hatchery, Washington (Figure 1-2). The hatchery is located about 25 mi north of Bonneville Dam. Fish were transported daily from the hatchery in a 100 gal fish transport tank mounted in a pick-up truck to the study site in lots of 100 to 400 fish. Fish were acclimated for about 24 h to the ambient water temperature of Columbia River at the study site prior to being tagged and released. The procedure used to gradually acclimate fish to the ambient river water was in accordance with the guidelines prepared by the U.S. Fish and Wildlife Service hatcheries. The difference in the two temperatures was generally less than 4.0^0C (7.2^0F). Ambient water temperature dropped from 17.0 to 14.5°C during the study. The fish transport tank was equipped with a recirculation system and supplemental oxygen supply. The transport time from the hatchery to the study site was less than two hours.

Treatment and control fish were drawn from the same group of fish assuring similar size and condition. Figure 2-1 shows the length frequency distribution of the treatment and control fish for the two treatment and control groups. The average length was almost identical for the three groups (123 to 125 mm total length).

2.2 Sample Size

One of the main considerations was to obtain an estimate of survival/fish condition within a specified precision level ($\varepsilon \leq \pm 0.05$, $\alpha = 0.10$) for each test condition using a relatively small number of fish (sample size). Because the objective of the study was not to delineate statistical differences ($P<0.05$) of specific magnitude between the two treatment conditions sample size calculations were not made for that purpose. Undoubtedly, the sample size requirements would be higher than calculated herein even if one desires to detect a modest difference, say ≥ 4%, between the two test conditions with a reasonable statistical power ($1-ß=0.80$) at $\alpha=0.05$ (RMC *et al.* 1994). Statistical power is a function of α, treatment effect size to be detected, sample size, and variance. Statistical significance (α) and treatment effect size to be detected can be pre-specified by investigators, estimate of variance needs to be obtained empirically or from other studies. However, should a need arise in the future to perform such calculations the estimates of the present study can be used.

The sample size is a function of the recapture probability (P), passage survival () or mortality (1-), survival probability of control fish (S), and the desired precision (α)at a given probability of significance (α). In general, sample size requirements decrease with an increase in control survival and recapture probabilities. Figure 2-2 shows an example relationship of control survival and recapture

probabilities and the sample size for achieving a precision ($\varepsilon \leq \pm 0.05$, $\alpha = 0.10$). In the present case, results of some previous survival investigations at hydroelectric dams on the Snake River and the Columbia River (RMC and Skalski 1994a,b; RMC *et al.* 1994; Normandeau Associates *et al.* 1995) provided preliminary estimates on the recapture and control survival probabilities to calculate sample sizes for this study. The derivation of precision is shown in Appendix A.

At the Rocky Reach Dam on the Columbia River, recapture probabilities of treatment fish were 0.885 to 0.955 and for control 0.935 to 0.988; control survival 0.935 to 0.988; and the immediate survival was 0.94 to 0.96 (RMC and Skalski 1994a,b). It was concluded that a sample size of 250 fish each for treatment and control groups would be adequate for achieving a precision of $\varepsilon \leq \pm 0.05$, ($1-\alpha = 0.90$) on the survival estimate. In the 1994 study at Lower Granite Dam the recapture probabilities for the treatment and control groups were 0.945 and 0.988, respectively, and the estimated immediate (1 h) passage survival () was 0.946 (RMC *et al.* 1994). In the 1995 survival research at Lower Granite Dam, the recapture probabilities for the treatment and control groups exceeded 0.96 (range of 0.964 to 0.996), with immediate (1 h) passage survival probabilities ranging from 0.946 to 0.975 (Normandeau Associates *et al.* 1995). The study also concluded that a shared control release for two treatment releases was a viable experimental protocol. Using the range of values obtained in the above studies and the variance expressions given in Appendix A, potential sample sizes were calculated (Table 2-1). We assumed a control survival (S) probability of 0.95 or 0.99, recapture probabilities (P) of 0.90, 0.95, and 0.99, passage survival (τ) of 0.95, 0.90, 0.925, and 0.85 or passage mortality (1-) of 0.05, 0.075, 0.10, and 0.15. These calculations showed that a precision of $\varepsilon \leq \pm 0.05$ on the point estimate of survival at alpha (α)=0.10 (the confidence level specified by ACOE) for each test condition could be achieved with a paired release of 311 fish if the expected passage survival is 0.925 and recapture probability is 0.90, and control survival is 0.99 (Table 2-1). With control survival probability of 0.95, recapture probability of 0.95, and expected passage survival of 0.925, a sample size of 284 fish each for treatment and control group would be needed (Table 2-2). It was further assumed that since the results of daily releases would be readily available, sample sizes could be adjusted accordingly. As stated earlier, based on results from the Lower Granite Dam survival research, a single control release was made for two treatment releases to minimize the use of specimens.

2.3 Release Locations

The ACOE had identified two primary test conditions for estimates of differential fish survival and condition at the Bonneville Dam spillway. One test condition involved estimating fish passage survival and condition at spillbay 2 (without a flow deflector) and the second involved fish passage

survival and condition at spillbay 4, which is equipped with a flow deflector (Figure 1-3. The spill through each spillbay was about 12,000 cfs (equivalent to four dogs), a discharge normally used during the emigration period. Control fish were released downstream of the discharge from spillbay 2 also at a discharge of 12,000 cfs (Figure 2-4). Fish were released about 3 ft above the water surface.

The treatment fish for each experiment were released at a pre-specified fixed location. The depth and location of the release hose for the treatment fish was determined in consultation with the ACOE personnel so that the fish would exit into a flow travelling about 5 ft/sec (Figure 2-3). The release hose was mounted so its terminus was 2 ft upstream of the regulating gate and 10 ft above the bottom of the gate (Figure 2-3). The hose was also mounted so that it stayed at this location when the gate was raised 6.8 ft above the sill to release 12,000 cfs spill (Figure 2-4).

Prior to initiating the full-scale study a "shake-down" was conducted. This involved releasing 10 treatment fish through each spillbay and 10 controls to identify any snags in the experimental procedures and protocols, safety concerns, expected recapture rates, and where the tagged fish were expected to surface. Upon satisfactory completion of the "shake-down" the full scale study was initiated on 10 October 1995.

In addition to the primary test conditions, the ACOE was also interested in obtaining a general idea on potential fish passage problems, if any, associated with ice and trash sluices at the two powerhouses (Figure 1-2). Consequently, a limited number of fish was also released through the sluices at Powerhouses 1 and 2. No control fish were released for these tests (Figure 2-5). A head gate for Powerhouse 2 sluice was lowered 5 ft below forebay elevation that provided approximately 650 cfs discharge. Fish were released about 2 ft below the water surface. The estimated discharge rate at Powerhouse 1 sluice was 200 to 300 cfs. Fish were released aty the water surface. Because of the limited scope, results of these releases are presented separately in Section 3.2.

2.4 Tag and Release

Tagging and release techniques were similar to those described for estimating direct effects of turbine or spill passage in Heisey *et al.* (1992) and RMC and Skalski (1994a,b). Fish were anesthetized in 0.5% MS222, held in a 4 gal tub and tagged with two HI-Z tags and a miniature radio tag. Additionally, each fish was given a uniquely numbered visual implant VI tag (Northwest Marine Technology, Inc., Shaw Island, Washington), for tracking survival and condition of individual smolts held over the 48 h period. When fish were fully recovered from anesthesia they were individually placed into the induction system holding tub (Heisey *et al.* 1992), tags activated, and fish released. Tagging crews were rotated daily so that each team released similar numbers of treatment and control fish. Normally a tagging crew tagged and released a total of 40 to 60 treatment or control fish on each day.

The treatment fish for both test conditions were released through an induction apparatus at a fixed location (Figures 1-3 and 2-3) in each spillbay at a constant spill volume. Each induction apparatus consisted of a small holding basin attached to a 4 in diameter induction hose line and was supplied with ambient river water to ensure that fish were transported quickly within a continuous flow of water.

Lots of 5 to 10 treatment and control fish were alternately released throughout the day. Fish were selected randomly from each day's transport. We released 280 treatment fish for each test condition, along with a single matching control of 280 fish release for both treatment groups. This release scheme had proved logistically effective in a recent study at the Lower Granite Dam and utilized a smaller number of fish without sacrificing precision (Normandeau Associates *et al.* 1995). Thus, for the two studied test conditions, we released 560 treatment and 280 control fish. All fish releases were made during the daylight hours.

Downstream of spillbay 2 was chosen for control releases based upon discharge patterns, safety, and logistics of positioning the fish delivery hose (Figure 2-4). Discharge from spillbay 1 was initially planned so that both treatment and control fish released in the vicinity of spillbay 2 would travel downstream nearly parallel to the shore. However, discharge from spillbay 1 was believed to interfere with the attraction flow for the fish ladder and potentially impede the upstream passage of adult salmonids. A 3,000 cfs spill from spillbay 2 during testing of spillbay 4 (12,000 cfs) appeared to help keep fish away from the shore.

2.5 Fish Recapture

Shortly after release (generally two to five minutes) the tags inflated and buoyed the fish to the surface for rapid recapture by a recovery boat crew. Both treatment and control fish were retrieved from the tailwater by up to four boat crews. Recovery boat crews were notified of the radio tag frequency of each fish upon its release. To minimize crew bias, no crew was specifically assigned to retrieve either control or treatment fish (Mathur *et al.* 1996a). Only crew members trained in fish handling retrieved tagged fish.

The ACOE secured the services of personnel from the Department of Agriculture to scare the gulls from the tailrace. Hazing of gulls was to minimize the potential loss of buoyed experimental fish thus maintaining the use of pre-specified sample sizes. However, no gull predication, if any, was observed on experimental fish.

Radio signals were received on a 5-element Yagi antenna coupled to an Advanced Telemetry systems programmable scanning receiver. The radio signal transmission enabled the boat crew(s) to follow the movement of each fish after spillway passage and position the boat for quick retrieval when the balloon tag buoyed the fish to the surface. The boats maintained a safe distance downstream of the

turbulent water in the spillbay (Figure 2-4). For safety reasons, spill was curtailed to recapture buoyed fish that became entrapped in turbulent areas for more than 15 min. Fish with active radio tags that failed to surface were tracked for 30 minutes and then periodically to ascertain if fish were displaying movement patterns typical of emigrating smolts or that of a predator. Buoyed fish were retrieved and placed into an on-board holding facility. The tag(s) were removed by a pin puller (modified pliers). Each fish was examined for scale loss and injuries and assigned codes relative to descriptions presented in Table 2-3. Fish were also checked for the presence of a VI tag. If the VI tag was not obvious the fish was identified by a pectoral or caudal fin clip. Tagging and data recording personnel were notified via a two-way radio system of each fish's recovery time and condition.

Each recaptured fish was immediately examined for physical injuries. Because controlled experiments replicating and correlating each injury type/characteristics to a specific causative mechanism are lacking, a definitive classification of observed injuries in the field, particularly in the case of multiple injuries, is difficult (Eicher Associates 1987). Thus, only probable causes could be attributed to the observed fish injuries.

All fish recaptured alive were transferred in covered 5 gal pails as soon as possible to 600 gal holding pools located on an upper deck of the spillbay. Each day's treatment and control fish were held in the same pool for 48 h. Pools were continuously supplied with ambient river water and shielded to prevent fish escapement and potential avian predation.

2.6 Classification of Recaptured Fish

Recaptured fish and inflated tags recovered dislodged from fish were classified as described in Normandeau Associates *et al.* (1995) to estimate the immediate (1 h) and 48 h effects after passage. Immediate status of each fish was designated alive, dead, predation, or unknown. The following criteria were used to make these designations: (1) alive--recaptured alive and remained so for 1 h; (2) alive--when the fish did not surface but radio signals indicated movement patterns typical of emigrating juveniles; (3) dead--recaptured dead or dead within 1 h of release; (4) dead--when only inflated tag(s) without fish are recovered and telemetric tracking (stationary signals) or the manner in which tags surfaced not indicative of predation; (5) unknown--when nothing is recaptured and the exact status cannot be ascertained from the radio signals; (6) predation--when fish are either actually observed being preyed upon, predator is buoyed to the surface, or subsequent radio telemetric tracking and/or tag indicate predation (i.e., rapid movements of tagged fish in and out of turbulent waters or sudden appearance of fully inflated tags).

Mortalities which occurred >1 h after fish were released through the induction apparatus were assigned a status delayed mortality (48 h). However, fish held in pools were observed approximately at

12 h intervals. Dead fish were identified by the numbered VI tag, thoroughly examined for scale loss and injury, and necropsied to determine the potential cause of death. Additionally, all specimens alive at 48 h were anesthetized and closely examined for injury and descaling. Injury and descaling were categorized by type, extent, and area of body. This re-examination of immobilized fish minimized additional handling stress immediately upon recapture. The amount of descaling for each fish recorded during the detailed examination provided a better estimate than that noted immediately upon recapture. Criterion used for descaling was in accordance with the procedure used by the ACOE for smolt monitoring. Injuries were recorded at the initial examination upon recapture and the detailed examination at 48 h. This procedure was followed because some injuries, such as bleeding, were no longer evident at 48 h and some additional injuries were detected during the detailed examination.

2.7 Assumptions

The following explicit assumptions were made for application of the HI-Z tag in obtaining a valid estimate of injury/mortality rate: tagging, handling, and release do not differentially affect survival of treatment and control fish groups; treatment and control groups are equally vulnerable to recapture; and recovery crews do not differentially retrieve treatment and control groups. Statistical analyses were also performed to support the viability of these assumptions (RMC and Skalski 1994a,b).

2.8 Data Analysis

Passage survival rates of fishes are estimated using paired release-recapture methods (Ricker 1975; Burnham *et al.* 1987). Unlike earlier investigations, however, recaptures of both alive and dead fish are possible with the HI-Z tag-recapture technique (Heisey *et al.* 1992). Thus, parameters associated with both alive and dead fish can be incorporated into the construction of a statistical model. This, along with high recapture probabilities, can be used to precisely estimate passage survival rates (Mathur *et al.* 1996a). Survival estimates were calculated for each release scheme.

The following terms are used in the equations and likelihood functions that follow:

R_c = Number of control fish released,

R_T = Number of treatment fish released,

a_c = Number of control fish recaptured alive,

d_c = Number of control fish recaptured dead,

a_T = Number of treatment fish recaptured alive,

d_T = Number of treatment fish recaptured dead,

S = Probability fish survive from the release point of the controls to recapture,

P_A = Probability an alive fish is recaptured,

P_D = Probability a dead fish is recaptured,

= Probability a treatment fish survives to recapture point (*i.e.*, passage survival),

1- = Passage mortality.

The joint likelihood for the passage-related mortality (Skalski 1992) is as follows:

$$L(S, t, P_A, P_D | R_C, R_T, a_C, d_C, a_T, d_T) =$$

$$\binom{R_c}{a_c, d_c} (SP_A)^{a_c} ((1-S)P_D)^{d_c} (1 - SP_A - (1-S)P_D)^{R_c - a_c - d_c}$$

$$x \binom{R_T}{a_T, d_T} (St\ P_A)^{a_T} ((1-St)P_D)^{d_T} (1 - St\ P_A - (1-St)P_D)^{R_T - a_T - d_T} . \qquad \textbf{(1)}$$

The likelihood model is based on the following assumptions: (a) the fate of each fish is independent; (b) the control and treatment fish come from the same population of inference and share the same natural survival probability, S; (c) all alive fish have the same probability, P_A, of recapture; (d) all dead fish have the same probability, P_D, of recapture; and (e) passage survival (τ) and natural survival (S) to the recapture point are conditionally independent.

The above likelihood model has four parameters (P_a, P_D, S, τ) and four minimum sufficient statistics (a_c, d_c, a_T, d_T). The estimators associated with the above likelihood model are:

$$\hat{t} = \frac{a_T\ R_c}{R_T\ a_c}$$

$$\hat{S} = \frac{R_T\ d_c\ a_c - R_c\ d_T\ a_c}{R_c\ d_c\ a_T - R_c\ d_T\ a_c}$$

$$\hat{P}_A = \frac{d_c a_T - d_T a_c}{R_T d_c - R_c d_T}$$

$$\hat{P}_D = \frac{d_C a_T - d_T a_C}{R_c a_T - R_T a_c}.$$

The variance (Var) and standard error (SE) of the estimated passage mortality ($1-\hat{t}$) or survival ($\hat{t}$) are:

$$Var(1-\hat{t}) = Var(\hat{t}) = \frac{\hat{t}}{SP_A}\left[\frac{(1 - S\hat{t}P_A)}{R_T} + \frac{(1 - SP_A)\hat{t}}{R_c}\right],$$

$$SE(1-\hat{t}) = SE(\hat{t}) = \sqrt{Var(1-\hat{t})}.$$

The 90% confidence intervals on the estimated survival for each test condition were calculated using the profile likelihood method (Hudson 1971). The profile likelihood method constructs confidence intervals without the need to assume normality of the parameter estimates and are generally assumed superior to the normal approximations.

A likelihood ratio test was used to determine whether recapture probabilities were similar for dead (P_D) and alive (P_A) fish for each test condition (RMC and Skalski 1994a,b). The statistic tested the null hypothesis of the simplified model (H_O:P_A=P_D) versus the alternative of the most generalized model (H_A:P_A *NE* P_D). Depending upon the outcome of this analysis the parameters and their associated variances can be calculated using that model. The model outputs are provided in Appendix A.

Because the two test conditions (spillbay with a flow deflector and spillbay without a flow deflector) were studied concurrently with a single shared control group, a modification to likelihood model (1) was used to take into account dependencies within the study design. For any two treatment groups (denoted T_1 and T_2), the modified likelihood model is as follows:

$$L(S, t, b, P_A, P_D | R_C, R_{T_1}, R_{T_2}, a_C, d_C, a_{T_1}, d_{T_1}, a_{T_2}, d_{T_2}) =$$

$$\binom{R_c}{a_c, d_c} (SP_A)^{a_c}((1-S)P_D)^{d_c}(1-SP_A-(1-S)P_D)^{R_c - a_c - d_c}$$

$$x\binom{R_{T_1}}{a_{T_1}, d_{T_1}} (St\ P_A)^{a_{T_1}}((1-St)P_D)^{d_{T_1}}(1-St\ P_A-(1-St)P_D)^{R_{T_1} - a_{T_1} - d_{T_1}}$$

$$x\binom{R_{T_2}}{a_{T_2}, d_{T_2}} (S\,t^{e^b} P_A)^{a_{T_2}}((1-S\,t^{e^b})P_D)^{d_{T_2}}(1-S\,t^{e^b} P_A-(1-S\,t^{e^b})P_D)^{R_{T_2} - a_{T_2} - d_{T_2}} . \quad \textbf{(2)}$$

This likelihood has the same assumptions as model (1) and has five parameters (S, τ, ß, P_A, and P_D) that can be estimated. The survival rate for treatment T_1 is estimated by and for treatment T_2, by t^{e^b}. A likelihood ratio test with 1 degree of freedom was used to test for equality in survival rates between treatments T_1 and T_2 based on the hypothesis H_O:ß=0 versus H_a:ß *NE* 0. A likelihood ratio test was performed to compare recapture probabilities and reduce the dimensionability of the model and hence, improve precision if P_A=P_D.

For each test condition, chi-square analyses were performed to detect homogeneity (P=0.05) within the treatment and control trials with respect to recapture rates of alive, dead, and non-recovered fish. Results of the statistical analyses, along with the derivation of variance and precision, are given in Appendix A. Summarized results are discussed in the text.